CHARM SCIENCES, INC.
ROSA FAST5 DON QUANTITATIVE TEST

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GENERAL INFORMATION

ROSA FAST5 DON Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology. DON (deoxynivalenol or vomitoxin) is extracted from the samples using water. DON interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the Charm EZ-M reader and interpreted as parts per billion (ppb) or parts per million (ppm) DON. To convert results in ppb to ppm divide by 1000 (e.g., 5000 ppb = 5 ppm).

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding the Mycotoxin Handbook, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	*Charm Sciences, Inc.* 978-687-9200
Test Kit Name:	ROSA FAST5 DON Quantitative Test
Product Number:	LF-DONQ-FAST5
Effective Date of Instructions:	06/24/2016
Instructions Revision Number	3
Conformance Range:	0.5 – 5 ppm
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Wheat, corn, barley, distillers dried grain w/solubles (DDGS), malted barley, milled rice, oats, rough rice, sorghum, wheat bran, wheat flour, and wheat middlings
Extraction method:	For samples ground so that at least 90% passes a No. 20 sieve, shake vigorously 50 grams ground sample with 250 milliliters (mL) of deionized or distilled water for 1 minute. For samples ground so that 60 – 89% passes a No. 20 sieve, shake vigorously 50 grams of the ground sample with 250 mL of deionized water for 3 minutes.
Test Format:	Lateral flow strip
Detection Method:	Charm EZ-M reader, Model LF-ROSA-EZ-M

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Test Strips:

Remove from the container only the number of test strips to be used in 1 day, document time of removal. Keep these test strips at room temperature during daily use for up to 12 hours and unused test strips should be discarded.

b. DONQ-FAST5 Dilution Buffer:

(1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.

(2) Use pre-dispensed buffer tubes and buffer solution at room temperature (18 to 30 °C).

c. Negative Control:

DONQ-FAST5 Dilution Buffer is used as a negative control in TEST PROCEDURES section.

d. Positive Control:

(1) Reconstitute the dry positive control (provided with test kit) by adding 3.0 mL DONQ-FAST5 Dilution Buffer. Shake well; allow to stand for 10 minutes at room temperature before use, and mix again just before use. For storage see "**STORAGE CONDITIONS AND PRECAUTIONS" section on page 10.**

e. Reader and Test Strip Performance Testing:

(1) Equipment Setup

Charm EZ-M reader: Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test. Follow Charm EZ-M reader prompts to test calibration strips (LO CAL and HI CAL) and controls (NEG CTRL and POS CTRL). Select DONQ-FAST5 from the TESTS list if prompted.

(2) Test calibration strips daily to verify Charm EZ-M reader performance. Calibration strips must test/perform in the specified ranges.

(3) Test negative control and positive control weekly to verify test strip performance. Valid control ranges are:

(a) Negative Control: less than or equal to 100 ppb (0.1 ppm)

(b) Positive Control: 500 to 1500 ppb (0.5 to 1.5 ppm)

If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.

f. ROSA Incubator:

ROSA Incubator must be clean and level. The ROSA Incubator temperature must be at 45 ± 1 °C (the temperature indicator should match the incubator temperature).

EXTRACTION PROCEDURE

a. <u>Procedure for barley, corn, malted barley, milled rice, oats, rough rice, sorghum, wheat, wheat bran, and wheat flour:</u>

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 250 mL deionized or distilled water.

(3) For samples ground so that at least 90% passes a No. 20 sieve, shake vigorously for 1 minute. For samples ground so that 60 – 89% passes a No. 20 sieve, shake vigorously for 3 minutes.

(4) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(6) Repeat steps 1 to 5 for additional samples.

b. <u>Procedure for DDGS:</u>

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 250 mL deionized or distilled water.

(3) For samples ground so that at least 90% passes a No. 20 sieve, shake vigorously for 1 minute. For samples ground so that 60 – 89% passes a No. 20 sieve, shake vigorously for 3 minutes.

(4) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(5) Transfer 10 to 15 mL settled extract into a clean conical tube and label.

(6) Adjust pH of settled extract by adding 10-30% KOH dropwise until pH is 6.5 to 7.5. Monitor pH with pH strips or pH meter.

(7) Transfer 1 to 1.5 mL pH adjusted extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(8) Repeat steps 1 to 7 for additional samples.

c. <u>Procedure for wheat middlings:</u>

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add 250 mL deionized or distilled water.

(3) For samples ground so that at least 90% passes a No. 20 sieve, shake vigorously for 1 minute. For samples ground so that 60 – 89% passes a No. 20 sieve, shake vigorously for 3 minutes.

(4) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(5) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(6) Filter centrifuged extract by drawing into syringe and passing through GF/CA filter (purchased separately). Collect filtered extract in a clean micro-centrifuge tube and label.

(7) Repeat steps 1 to 6 for additional samples.

SAMPLE PREPARATION FOR QUANTIFICATION

a. <u>Sample Preparation of Diluted Extract for 0.5 to 1.0 ppm quantitation.</u>

(1) Pipet 1.0 mL DONQ-FAST5 Dilution Buffer into a clean micro-centrifuge tube.

(2) Pipet 100 microliters (µL) centrifuged or filtered sample extract to micro-centrifuge tube containing 1.0 mL DONQ-FAST5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This sample is the Diluted Extract, and ready for the test (use within 6 hours after preparation).

(3) Repeat for additional samples.

b. <u>Sample Preparation of Second Diluted Extract for 0.8 to 5 ppm quantitation.</u>

(1) Pipet 1.0 mL DONQ-FAST5 Dilution Buffer into a clean micro-centrifuge tube.

(2) Pipet 300 µL Diluted Extract to micro-centrifuge tube containing 1.0 mL DONQ-FAST5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label. This sample is the Second Diluted Extract, and ready for the test (use within 6 hours after preparation).

(3) Repeat for additional samples.

NOTE: Laboratories may initially test the Second Diluted Extract if levels typically reported in their market area are within the 1.0 to 5 ppm testing range.

TEST PROCEDURES

a. <u>Sample Analysis:</u>

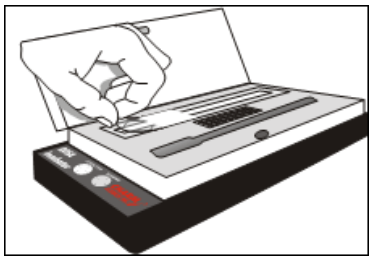
(1) Check that the ROSA Incubator temperature is 45 ± 1 °C.

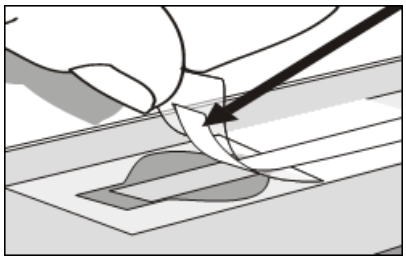
(2) Label test strip(s) to identify sample.

(3) Place test strip in the ROSA Incubator with the flat side facing upward.

(4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to "Peel to Here" line.

Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.





(5) Hold the pipet vertically and slowly pipet 300 µL test sample (diluted extract or positive and negative control) into the sample compartment at the ROSA Incubator line.

(6) Reseal the tape over the sample pad compartment.

NOTE: When performing multiple tests using a ROSA Incubator:

(a) Peel, pipet, and reseal before starting next strip.

(b) Complete all test strips within 1 minute.

(7) Close lid on the ROSA Incubator.

(8) Incubate for 5 minutes.

(9) Remove strip from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

(a) Wipe foreign matter (dust, etc.) from the test strip(s).

(b) Inspect and read test strip(s) within 2 minutes of incubation completion. When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time.

(c) Lower ROSA Incubator lid; do not re-latch.

b. <u>Visual Inspection:</u>

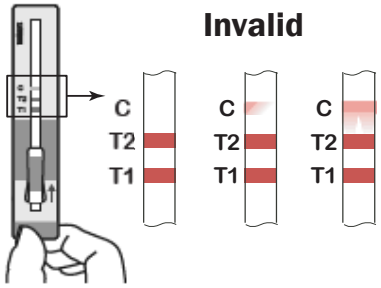
(1) The test strip is **INVALID** if any of the following are observed:

(a) C (Control) line is missing.

(b) T1, T2 (Test) or C line is smeared or uneven.

(c) T1, T2, or C line is obscured by diluted extract or control.

(d) Beads do not flow past T1, T2 or C lines.



(2) Do not put INVALID test strips in the Charm EZ-M reader.

(3) If test strip is INVALID, re-test the diluted extract or control.

c. <u>Interpretation:</u>

(1) Insert a clean and valid test strip into the Charm EZ-M reader (read only mode). Slide the strip into the slot with the sample compartment in the down position until it stops.

(2) Due to an issue observed with analysis of the Diluted Extract, the quantitative range of the Diluted Extract was changed from 0.5 – 1.5 ppm to 0.5 – 1.0 ppm and the range for the Second Diluted Extract was changed from 1.0 – 5 ppm to 0.8 – 5 ppm in this version of the test kit instructions.

Read results on DONQ-FAST5 from the TESTS list with COMMODITY and DILUTION selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.

- **DE:** Diluted Extract for 0.5 to 1.0 ppm quantitation.
- **2ND DE:** Second Diluted Extract for 0.8 to 5 ppm quantitation.
- **SUPP DE:** Supplemental Diluted Extract for 1 to 5.4 ppm (Uncorrected DON Concentration) quantitation.

Note: For controls, see Reader and Test Strip Performance Testing in PREPARATION OF TEST MATERIALS AND EQUIPMENT section.

(3) **READING:** The number displayed is the concentration of DON (ppb or ppm) in the sample. A reading in ppb must be converted to ppm by dividing the ppb concentration by 1000 (e.g., 500 ppb = 0.5 ppm).

If the result from the Diluted Extract is greater than 1.0 ppm, the Second Diluted Extract must be prepared and analyzed for quantitation from 0.8 – 5 ppm (5.4 ppm if reporting results to the tenths).

Analysis of the Second Diluted Extract covers from 0.8 – 5 ppm (5.4 ppm if reporting results to the tenths). If less than 0.8 ppm, preparation and analysis of the Diluted Extract must be performed.

A Second Diluted Extract READING greater than 5 ppm (5.4 ppm if reporting results to the tenths) indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. An applicant can request a supplemental analysis option to report test results above the Second Diluted Extract sensitivity range of 5 ppm (5.4 ppm if reporting results to the tenths). See SUPPLEMENTAL ANALYSIS section for more information.

Note: Applicants may request qualitative certification in lieu of retesting of results outside of the Diluted or Second Diluted Extract test sample sensitivity ranges/concentrations.

SUPPLEMENTAL ANALYSIS

Supplemental analysis is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative DON test kits is 0.5 to 5 ppm. Therefore, supplemental analysis would be performed for a result above 5 ppm (5.4 ppm if reporting results to the tenths). In supplemental analysis, the Second Diluted Extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range, and a correction for dilution is applied to derive the final result. For this test kit, the READING is an Uncorrected DON Concentration in the sample and the Corrected DON Concentration is obtained by multiplying the Uncorrected DON Concentration by the dilution factor used to prepare the Supplemental Diluted Extract.

Supplemental analysis is performed only at the request of the applicant.

Preparation and Assay of Supplemental Diluted Extract.

(1) Prepare Second Diluted Extract according to Sample Preparation for Quantification.

(2) Determine and record the Dilution Factor (DF) required to prepare Supplemental Diluted Extract for the Suspected Sample Concentration. The Dilution Factor (see equation below) is equal to the sum of the volume of the DONQ-FAST5 Dilution Buffer plus the volume of the Second Diluted Extract divided by the volume of the Second Diluted Extract. See table below for examples.

$$\mathbf{DF} = \frac{\text{Dilution Buffer Volume (in mL)} + \text{Second Diluted Extract Volume (in mL)}}{\text{Second Diluted Extract Volume (in mL)}}$$

DF	DONQ-FAST5 Dilution Buffer Volume	Second Diluted Extract Volume	Suspected Sample Concentration
4.3	1.0 mL	0.3 mL (300 µL)	4 to 20 ppm
11	1.0 mL	0.1 mL (100 µL)	11 to 60 ppm

(3) Prepare Supplemental Diluted Extract from the Second Diluted Extract.

(a) Pipet determined volume of DONQ-FAST5 Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet the determined volume of Second Diluted Extract to micro-centrifuge tube containing DONQ-FAST5 Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the Supplemental Diluted Extract.

(4) Repeat steps 1 to 3 for additional samples.

(5) Use Supplemental Diluted Extract as test sample in Sample Analysis found in TEST PROCEDURES section.

(6) Inspect and interpret the test strip as directed in TEST PROCEDURES section.

A reading greater than 5.4 ppm indicates that the concentration of the sample is greater than the test range. Prepare another Supplemental Diluted Extract with a higher Dilution Factor and run another test strip to quantitate.

NOTE: The number/result displayed is the Uncorrected DON Concentration in the sample.

(7) Multiply the result by the Dilution Factor used to prepare the Supplemental Diluted Extract to convert the Uncorrected DON Concentration to the final Corrected DON Concentration.

Example: If the Uncorrected DON Concentration is 2.0 ppm and the Dilution Factor is 11 the final Corrected DON Concentration is 22 ppm (2.0 ppm x 11 = 22 ppm).

A final result less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5 ppm.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions:

(1) Store test strips refrigerated in tightly closed supplied container.

(2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.

(3) Store reconstituted positive control refrigerated (0 to 7 °C) for up to 1 week or aliquot (at least 0.5 mL) to clean micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution (-15 °C or below) for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed positive control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

b. Precautions:

(1) Test Strips

(a) To open test strip canister, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.

(b) In high humidity, limit condensation by opening container after it has warmed to room temperature, estimated between 20 to 30 minutes from the time the container was removed from the refrigerator.

(c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if the blue beads have turned purple or pink

(2) Use DONQ-FAST5 Dilution Buffer supplied with each test kit only.

(3) Do not use the test kits beyond the noted expiration date.

(4) Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.

(5) ROSA Incubator must be clean and level. ROSA Incubator temperature must be 45 ± 1 °C. The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched

unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.

EQUIPMENT AND SUPPLIES

a. <u>Test Strips</u>

(1) LF-DONQ-FAST5-20K

(a) 1 container of 20 DONQ-FAST5 test strips

(b) 1 1000 ppb DON Positive Control

(c) 1 DONQ-FAST5 Dilution Buffer

(2) LF-DONQ-FAST5-100K

(a) 1 container of 100 DONQ-FAST5 test strips

(b) 1 1000 ppb DON Positive Control

(c) 2 DONQ-FAST5 Dilution Buffers

(3) LF-DONQ-FAST5-500K

(a) 5 containers of 100 DONQ-FAST5 test strips

(b) 5 1000 ppb DON Positive Controls

(c) 10 DONQ-FAST5 Dilution Buffers

b. <u>Materials required but not provided</u>

(1) 100 µL pipet and pipet tips

(2) 300 µL pipet and pipet tips

(3) 100 to 1000 µL variable volume pipet or 1.0 mL pipet and pipet tips

(4) 250 mL graduated cylinder

(5) Balance

(6) Deionized or distilled water

(7) Micro-centrifuge tubes

(8) Mini-centrifuge

(9) Charm EZ-M reader

(10) Printer for Charm EZ-M reader (optional)

(11) ROSA Incubator

(12) Sample extraction containers or Whirl-pak bags

(13) Sample grinder

(14) Storage bottle

(15) Transfer pipets (optional)

c. <u>Materials required but not provided for testing distillers dried grain with solubles</u>

(1) 10-30% KOH (w/v) in water

(2) Conical tubes

(3) pH paper or pH meter

d. <u>Materials required but not provided for wheat middlings</u>

(1) GF/CA syringe filters (Phenomenex Part No. AF0-8A09-12)

(2) Syringes

REVISION HISTORY

Revision 3 (06/24/2016)

- Changed extraction procedure. Samples ground to 90% passing a No. 20 sieve are extracted by shaking for 1 min. Samples ground to 60 – 89% passing a No. 20 sieve are extracted by shaking for 3 minutes.
- The words "(e.g., grinding and dividing)" were taken out from the 3rd paragraph of "**GENERAL INFORMATION"** section on page 1

Revision 2 (12/04/2015)

- Procedure for mixing the diluted extract was updated.
- Flow chart was added.

Revision 1 (10/8/2015)

- Due to an issue observed with analysis of the Diluted Extract, the quantitative range of the Diluted Extract was changed from 0.5 – 1.5 ppm to 0.5 – 1.0 ppm and the range for the Second Diluted Extract was changed from 1.0 – 5 ppm to 0.8 – 5 ppm.

Revision 0 (2/2/2015)

FLOW CHART

Will be provided later